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Platelet-leukocyte aggregation during hemodialysis

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Platelet-leukocyte aggregation during hemodialysis. Hemodialysis is associated with simultaneous changes in leukocytes and platelets, but it is unclear whether these alterations affect the interactions between these cell types. To evaluate this process, we examined the appearance of platelet specific antigens (CD41) on leukocytes as an index of platelet-leukocyte aggregation during hemodialysis using three different synthetic membranes. Patients with end-stage renal disease (ESRD) on long-term hemodialysis treatment were enrolled. Flow cytometric techniques and platelet specific monoclonal antibodies (MoAb) that recognize the glycoprotein complex on resting and activated platelets (anti-CD41), the activated GPIIb-IIIa complex receptor (anti-LIBS1), and the p selectin GMP140, that is exposed on platelet plasma membrane after activation and platelet degranulation (anti-CD62), were used. Subjects with ESRD had a lower predialysis platelet surface expression of CD41 and LIBS1 compared to normal controls, but unchanged CD62 expression. In parallel, patients with ESRD manifested a uniformly reduced platelet-leukocyte microaggregates predialysis compared to normal controls. When examined across the dialyzer, however, an increase in platelet-neutrophil and platelet-monocyte microaggregates was observed with all three synthetic membranes at both 15 and 30 minutes after initiation of dialysis. This phenomenon could be duplicated *in vitro* by physiologic concentrations of the platelet specific agonist ADP, but not by the complement factors C3a or C5a. We conclude that platelet-leukocyte aggregates occur during dialysis likely related to a primary platelet activation mechanism. This phenomenon may serve as a new biocompatibility parameter and may shed light on some of the biologic consequences of hemodialysis.

Abnormalities in platelet function have been among the earliest hematological defects observed in uremia to attract intensive study [reviewed in 1]. These early studies focused on the elucidation of the mechanism of the defect in platelet function that underlies the enhanced bleeding tendency of the untreated uremic state and its correction by hemodialysis [1]. Another aspect of platelet function, however, namely platelet activation by hemodialysis, has received less attention except as an index of biocompatibility of dialysis membranes [2–4]. Platelet activation and interaction with plasmatic components play a key role in hemostatic mechanisms during hemodialysis treatment [2–4]. The role of platelets, however, is much broader than their hemostatic function. A dynamic interplay between platelets and circulating cellular elements has been observed under a variety of physiologic and pathologic states [5–10], and platelet interaction with endothelium offers the theoretical possibility of wide reaching effects of platelet activation [7, 11].

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Platelet activation occurs rapidly after onset of hemodialysis [4]. Because dialysis induced activation of blood platelets coincides with leukocyte activation [2, 4, 7], it is possible that platelet interaction with leukocytes occurs to a significant degree during hemodialysis. Such a phenomenon may play an important pathophysiological role in hemodialysis by facilitating platelet-endothelial interaction by the intermediary of activated leukocytes [7, 11]. Further, study of these interactions may provide an insight into the dynamic changes that characterize hemocompatibility aspects of the blood dialyzer interface. To date, co-investigation of the interplay between the different parameters of biocompatibility has centered on plasmatic-cellular interactions (such as complement and neutrophils). A study of platelet-leukocyte interactions would offer the novel aspect of cellular-cellular interactions as a possible parameter of hemocompatibility. Therefore, using flow cytometric techniques and platelet specific monoclonal antibodies we studied changes in platelet surface glycoproteins expression, and formation of platelet-leukocyte microaggregates during hemodialysis.

Methods

Patient population

Nine stable, end-stage renal disease (ESRD) patients, between 18 and 75 years old (mean ± SD, 48 ± 18), treated with hemodialysis for more than six months, on a stable anticoagulation regimen, hematocrit above 26%, and able and willing to give informed consent were entered in this study. The protocol of this study was reviewed and approved by the University Ethical Committee. Exclusion criteria included unstable clinical conditions, cardiac and vascular instability, positive history for first use syndrome, unstabilized erythropoietin dosage, or single needle dialysis. None of the patients was known to have pre-existing hemostatic disorders unrelated to uremia, infections, or had received any medications known to affect platelet function for at least two weeks prior to the study.

Study design

The study covered three weeks of treatment, with one week of treatment on each dialyzer. The dialyzer sequence was randomized. Blood samples were collected for analysis during the third treatment. The three dialyzers tested were Fresenius Hemoflow F60S (Polysulfone PS600, 1.3 m^2, steam sterilized), Hospal Filtral-10 AN69HF (PAN-methallylsulfonate, 0.85 m^2, ETO-sterilized) and Nikkiso GLX-18GW containing a new modified PAN-membrane with reduced negative charges (SPAN, 1.8 m^2, gamma sterilized).

Dialysis procedure

Bicarbonate dialysate was used in all treatments. Dialyzers were rinsed single pass with 2.0 liters of 0.9% NaCl solution containing 1000 IU heparin. Heparin was the sole anticoagulant used throughout all treatments. A patient's individual heparin regimen was maintained. A heparin bolus was administered systemically before connection with the extracorporeal circuit, and five minutes later the extracorporeal circuit was established and a continuous infusion of heparin was started. Blood flow rate, ultrafiltration volume, dialysate composition and duration of dialysis treatment was according to the patients individual prescriptions. Parameters were evaluated at baseline, 15, 30, and 240 minutes and samples obtained form the inlet and outlet sides of the dialyzer.

Monoclonal antibodies

All monoclonal antibodies (MoAb) used in this study were conjugated with fluorescein isothiocyanate (FITC) according to standard methods. MoAb anti-CD41 (Dianova) is a platelet-specific anti-GPIIb-IIIa MoAb that recognizes the glycoprotein complex on resting and activated platelets. Anti-LIBS1 (anti-GPIIIa, generously provided by Dr. Mark Ginsberg, Research Institute of Scripps Clinic, La Jolla, California, USA) is a conformation-dependent MoAb that preferentially binds to the activated GPIIb-IIIa complex receptor [12]. Anti-CD62 (Dianova) recognizes the p selectin GMP140, that is exposed on platelet plasma membrane after platelet activation and degranulation has occurred [13].

Flow cytometric analysis

Platelets. Flow cytometric (FACS) analysis of platelets was performed as previously described [4, 12]. All samples were drawn from the dialyzer inlet and outlet ports, anticoagulated with citrate (ACD, NIH formula) at a ratio of 1 volume ACD to 7 volumes of blood, and immediately placed on crushed ice. Platelet rich plasma (PRP) was obtained from ACD anticoagulated blood samples after sedimenting red and white blood cells at 180 × g for 20 minutes at 4°C. Thereafter, 5 μl of PRP aliquots were added to polypropylene tubes containing a saturating concentration of fluorescein (FITC) conjugated monoclonal antibodies in a total volume of 50 μl Tyrodes buffer (0.1% BSA, 0.1% glucose, 2 mM $MgCl_2$, 137.5 mM NaCl, 12 mM $NaHCO_3$, 2.6 mM KCl, pH 7.4). After a 30-minute incubation period at room temperature in the dark, 1 ml Tyrodes buffer was added to each vial and samples were stored on ice. FACS analysis was performed within one hour. Platelets were identified in the forward versus side scatter plot. The gated platelet population was found to bind > 96% the platelet specific monoclonal antibody anti-CD41.

Platelet-leukocyte interaction. Platelet-leukocyte interaction was evaluated according to published methods [10, 14] with minor modifications. In brief, MoAb anti-CD41 was used as a platelet specific marker to evaluate platelet-leukocyte interaction. To 25 μl of whole blood, 25 μl Tyrodes buffer containing saturating concentrations of MoAb anti-CD41 was added and incubated for 30 minutes at room temperature in the dark. Thereafter, red blood cells were lysed and fixed with SIGMA lysing reagent according to the manufacturer's protocol and kept on ice before analysis. For flow cytometric analysis leukocytes were identified by size and granularity in the forward versus side scatter plot. Leukocyte subgroups, polymorphonuclear granulocytes, monocytes, and lymphocytes were identified by use of subgroup specific MoAbs. The mean intensity of fluorescence of anti-CD41 of the leukocyte subgroups was used to characterize platelet-leukocyte interaction. Samples were analyzed on a FACScan cytometer (Becton Dickinson, Mountain View, California, USA) equipped with an argon laser. The instrument was calibrated twice weekly for fluorescence and lightscatter using 2 μm calibrite beads (Becton Dickinson). Five thousand particles were analyzed for each sample at a constant flow rate of 50 to 150 events per second. Logarithmic amplification was used for the fluorescence signal. Data were collected and analyzed on a Hewlett Packard computer equipped with FACScan software program.

In vitro studies. Platelet-leukocyte interactions were studied in whole blood obtained from five normal volunteers and six ESRD subjects and incubated with increasing physiologic concentrations of ADP. To 25 μl of whole blood, 5 μl of ADP solution and 20 μl Tyrodes buffer containing saturating concentrations of MoAb anti-CD41 were added and the mixture incubated for 15 minutes at room temperature in the dark. The concentration of ADP was calculated to achieve the final desired concentration of the agonist. Samples were then processed as described above. For platelet activation studies, platelet rich plasma (PRP) was obtained from ACD anticoagulated blood samples as described above. Thereafter, 5 μl of PRP aliquots were added to polypropylene tubes containing 5 μl of a saturating concentration of fluorescein (FITC) conjugated anti-LIBS1, 5 μl of ADP solution, and 35 μl Tyrodes buffer. After a 15-minute incubation period at room temperature in the dark, samples were processed as described above.

Analytical methods

Blood count was done using the Coulter System T840, (Coulter, Krefeld, Germany); C5a using Enzygnost C5a, (Behringwerke, Marburg, Germany).

Statistics

Statistical analysis was performed using the statistical package SPSS for Windows Vs.5.01, (SPSS Inc., Chicago, Illinois, USA). Groups were compared by analysis of variance followed by unpaired *t*-test for significant differences. Values reported are mean ± standard error of the mean.

Results

Surface expression of platelet glycoproteins

Effects of ESRD. Activation of circulating blood platelets was assessed by surface expression of platelet glycoproteins using MoAbs. Platelet release reaction was characterized by measuring plasma membrane expression of granule membrane protein GMP140 using anti-CD62. The appearance of the activated fibrinogen receptor on GPIIb-IIIa was assayed using a conformation dependent MoAb anti LIBS1, which preferentially recognizes a ligand induced epitope on GPIIIa [12].

Subjects with ESRD had a lower predialysis surface expression of CD41 [ESRD 794 ± 2 vs. normals 810 ± 4 (mean anti-CD41 fluorescence units), $P < 0.01$] and LIBS1 [ESRD 287 ± 10 vs. normals 414 ± 21 (mean anti-LIBS1 fluorescence units), $P < 0.01$] compared to normal controls, but unchanged CD62 expression

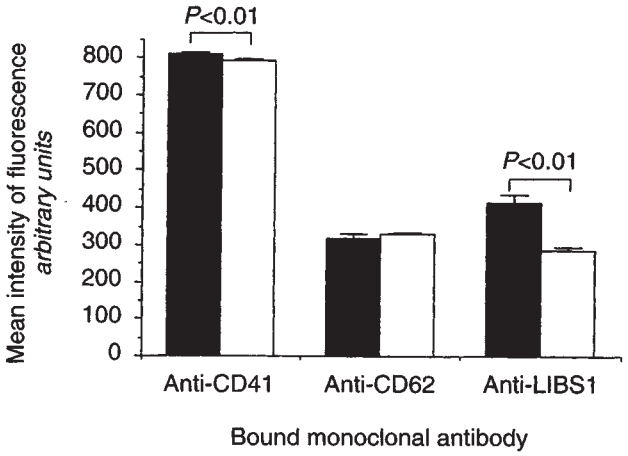

Fig. 1. *Expression of antigens CD41, CD62 and LIBS1 determined on non-stimulated platelets from normal subjects (■) and patients with ESRD (□) prior to dialysis session.* A significantly lower CD41 and LIBS1 expression were found in the uremic subjects.

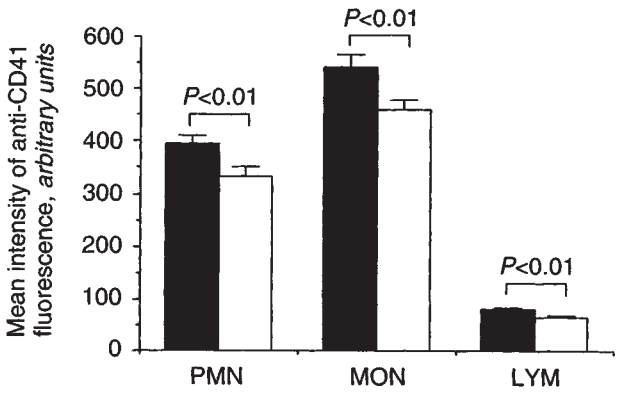

Fig. 2. *Formation of platelet-leukocyte aggregates.* Binding of platelet specific anti-CD41 MoAb was determined on leukocyte subgroups. Platelet-leukocyte aggregates were lower in ESRD patients (□) predialysis compared to normal controls (■). This was true for all 3 cell types (neutrophils, monocytes and lymphocytes). Abbreviations are: PMN, polymorphonuclear neutrophil; MON, monocytes; LYM, lymphocytes.

Table 1. Effect of hemodialysis on platelet surface expression of CD41, CD62 and LIBS1

		Time *min*			
		0	15	30	240
CD41	F60S	794 ± 3	794 ± 2	796 ± 2	791 ± 2
	PAN	798 ± 5	799 ± 4	799 ± 5	789 ± 4
	SPAN	796 ± 2	793 ± 4	796 ± 3	787 ± 4
CD62	F60S	317 ± 12	322 ± 15	317 ± 13	327 ± 16
	PAN	304 ± 7	318 ± 11	303 ± 20	321 ± 7
	SPAN	315 ± 15	323 ± 14	314 ± 10	333 ± 15
LIBS1	F60S	313 ± 26	304 ± 12	305 ± 9	292 ± 19
	PAN	290 ± 15	322 ± 16	285 ± 13	282 ± 10
	SPAN	273 ± 10	309 ± 16	304 ± 18	276 ± 13

No significant change with any membrane was observed in arterial platelet values for these parameters throughout dialysis. Values are mean intensity of fluorescence of the respective MoAbs.

[ESRD 329 ± 8, normals 317 ± 13, (mean anti-CD62 fluorescence units)] (Fig. 1).

Effect of hemodialysis. There was no change during dialysis in the surface expression of any of the platelet glycoproteins studied in the arterial blood samples suggesting no changes in the status of platelets in the systemic circulation (Table 1).

Platelet-leukocyte aggregates

Effect of ESRD. Formation of platelet-leukocyte microaggregates was studied by evaluating subpopulations of leukocytes (neutrophils, monocytes, lymphocytes) for binding of platelet specific anti-CD41. Patients with ESRD manifested a uniformly reduced platelet-leukocyte microaggregates predialysis compared to normal controls [PMN: ESRD 332 ± 18, normal 394 ± 18, monocytes: ESRD 460 ± 19, normal 541 ± 23, lymphocytes: 65 ± 2, normal 80 ± 4, (mean anti-CD41 fluorescence units), $P < 0.01$ for all 3 cell types] (Fig. 2).

Effect of hemodialysis. When examined simultaneously across the dialyzer, an increase in platelet-neutrophil microaggregates was observed with all three synthetic membranes at both 15 and 30 minutes after initiation of dialysis (Fig. 3). The mean increase anti-CD41 fluorescence units on neutrophils was for F60S 48, PAN 44, and SPAN 51. An identical increment was observed at 30 minutes of dialysis. A similar phenomenon was observed for platelet-monocyte microaggregates (Fig. 4). The mean increase anti-CD41 fluorescence units on monocytes was for F60S 54, PAN 36, and SPAN 79. An identical increment was observed at 30 minutes of dialysis. Lymphocyte platelet interaction was minimal and unaffected by passage across the dialyzer. When arterial values were examined over time, there was no increase in the systemic circulation in the prevalence of platelet-neutrophil, -monocyte or -lymphocyte aggregates during hemodialysis (Table 2).

To determine whether cellulosic membranes show a similar phenomenon, we evaluated seven patients being dialyzed routinely with Hemophan. When examined simultaneously across the dialyzer, an increase in platelet-neutrophil microaggregates was observed with Hemophan similar to the three synthetic membranes. At 15 minutes after initiation of dialysis there was a step up across the dialyzer for anti-CD41 binding to neutrophils from 410 ± 21 to 476 ± 24 ($P < 0.05$), and to monocytes from 546 ± 27 to 633 ± 19 ($P < 0.05$). The mean increase anti-CD41 fluorescence units on neutrophils and monocytes with Hemophan was similar to that observed with the three synthetic membranes.

Effect of ADP in vitro. To have some insight into the mechanism of the formation of platelet-leukocyte aggregates, the platelet specific agonist ADP was added in increasing concentrations to whole blood from five normal controls and the occurrence of platelet-leukocyte aggregates *in vitro* examined. A concentration dependent increase in platelet-neutrophil and platelet-monocyte aggregates was observed at concentrations of ADP within the physiologic range (Fig. 5). There was no change in platelet-lymphocyte aggregates. The effect was saturable at ADP concentration of 10 μM. A similar phenomenon was observed in leukocytes obtained from six subjects on hemodialysis, the peak response, however, was lower in uremics than that observed in normal control. At a concentration of ADP of 10 μM, the increase in anti-CD41 binding to neutrophils in normals was 248 ± 62, whereas it was 142 ± 68 in uremics (arbitrary fluorescence units, $P < 0.05$). Similarly, at a concentration of ADP of 10 μM, the increase in anti-CD41 binding to monocytes in normals was 296 ±

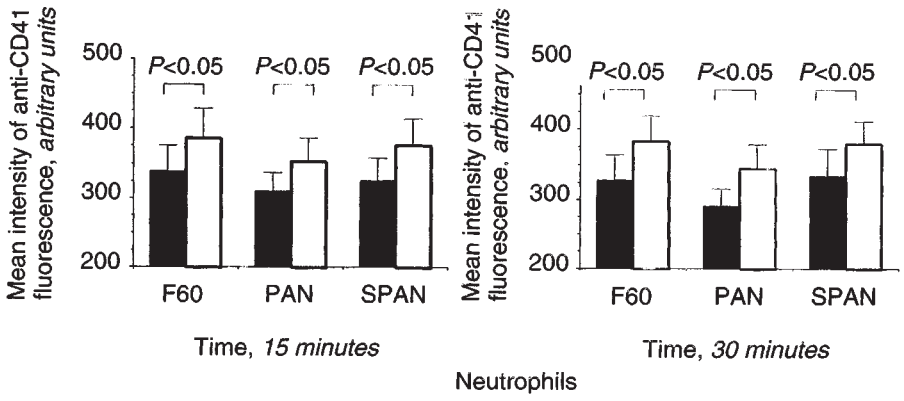

Fig. 3. *Effect of dialysis on platelet-neutrophil aggregates.* There was an increased formation of platelet-neutrophil aggregates across the dialyzer both at 15 and 30 minutes after the start of dialysis. Symbols are: (■) dialyzer inlet; (□) outlet. Abbreviations are: F60S, polysulphone; PAN, polyacrylonitrile; SPAN, specially modified polyacrylonitrile.

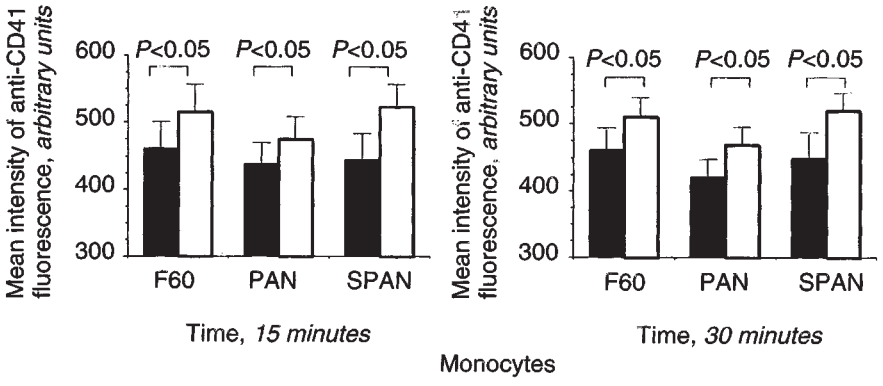

Fig. 4. *Effect of dialysis on platelet-monocyte aggregates.* There was an increased formation of platelet-monocyte aggregates across the dialyzer both at 15 and 30 minutes after the start of dialysis. Symbols are: (■) dialyzer inlet; (□) outlet. Abbreviations are: F60S, polysulphone; PAN, polyacrylonitrile; SPAN, specially modified polyacrylonitrile.

Table 2. Effect of hemodialysis on platelet-leukocyte aggregates

		Time *min*			
		0	15	30	240
PMN	F60	345 ± 36	340 ± 37	328 ± 36	333 ± 29
	PAN	315 ± 22	311 ± 27	290 ± 26	337 ± 27
	SPAN	338 ± 38	321 ± 37	333 ± 39	280 ± 25
MON	F60	486 ± 36	462 ± 39	462 ± 34	474 ± 38
	PAN	437 ± 27	440 ± 32	421 ± 28	451 ± 32
	SPAN	455 ± 36	445 ± 39	451 ± 38	422 ± 29

No significant change with any membrane was observed in arterial platelet-leukocyte aggregates throughout dialysis. Values are mean intensity of fluorescence of anti-CD41 on the respective cells. Abbreviations are: PMN, polymorphonuclear neutrophils; MON, monocytes.

37, whereas it was 183 ± 61 in uremics (arbitrary fluorescence units, $P < 0.05$). The formation of platelet-leukocyte aggregates in blood from normal controls very closely paralleled the profile of activation by ADP of the platelet fibrinogen receptor (Fig. 5). Addition of complement fragments (C3a, C5a) *in vitro* at high physiologic concentrations had no effect on platelet-leukocyte interactions (data not shown).

Biocompatibility parameters

A minimal non-significant decline in white cell count was observed at 15 minutes for all three synthetic membranes, but otherwise WBC counts were unchanged. Arterial platelet counts were unchanged throughout treatment for the three membranes. Dialyzer inlet C5a values, reflecting systemic status of the complement system were not altered by any of the three synthetic membranes. At every time point outlet values for C5a were higher than inlet for both F60 and SPAN, but not for PAN. The increase in C5a with F60 and SPAN is minimal and remains in the range expected for highly biocompatible synthetic membranes. Further, the high capacity of PAN to adsorb complement should be kept in mind when this parameter is used to compare different membranes.

Discussion

The results of the present study can be summarized as follows: (1) Subjects with ESRD had a lower predialysis surface expression of CD41 and LIBS1 compared to normal controls, but unchanged CD62 expression; (2) with the synthetic membranes used, there was no change during dialysis in the surface expression of any of the platelet glycoproteins studied in the arterial blood samples, suggesting no changes in the status of platelets in the systemic circulation; (3) patients with ESRD manifested a reduced platelet-leukocyte microaggregates predialysis compared to normal controls; (4) when examined simultaneously across the dialyzer, an increase in platelet-neutrophil and platelet-monocyte microaggregates was observed with all three synthetic membranes, but not for lymphocytes. Similar findings were obtained using a modified cellulosic membrane. This phenomenon could be duplicated *in vitro* by physiologic concentrations of the platelet specific agonist ADP, but not by the complement factors C3a or C5a. The occurrence of this phenomenon with membranes of differing complement activating potential and the inability to reproduce

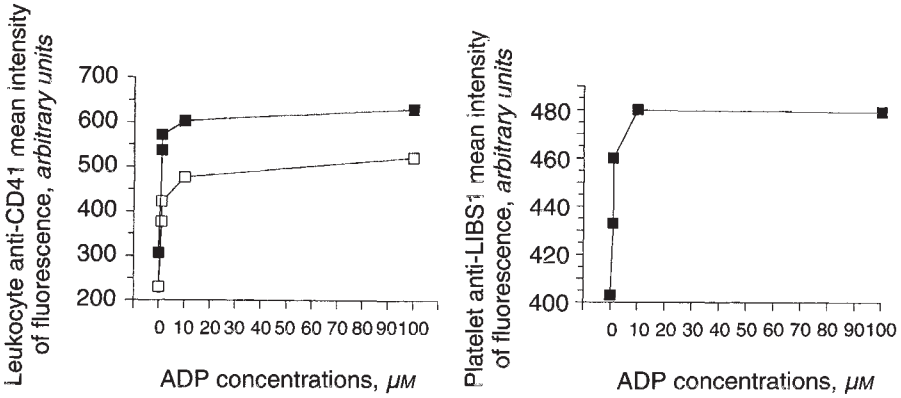

Fig. 5. *Whole blood from normal controls was stimulated with various concentrations of the platelet agonist ADP and platelet-leukocyte aggregation was determined as described in* **Methods**. In parallel, platelet rich plasma was stimulated with similar concentrations of ADP and platelet activation was assessed by anti-LIBS1 binding. ADP induced the formation of platelet-neutrophil and platelet-monocyte aggregates in parallel with platelet activation.

the finding *in vitro* with complement lead us to suggest that it appears to be complement independent.

Several defects in platelet function have been observed in uremia, but a consistent explanatory framework for the platelet functional impairment has yet to emerge [1]. The present study has identified novel aspects of platelet function in patients with ESRD independent of the effects of dialysis. Non-stimulated uremic platelets had a reduced surface density of glycoprotein GPIIb-IIIa (CD41) and less fibrinogen receptors in an active state (LIBS1) than non-stimulated platelets of control subjects, but the spontaneous release reaction (as reflected in CD62) of these circulating platelets is similar in uremics and controls. These results suggest a defect in the expression of the fibrinogen receptor on platelets in both its inactive and active forms (Gawaz et al, manuscript in preparation). The possible binding of depolymerized factor VIII present in uremic blood to the fibrinogen receptor would not lead to a reduction in platelet anti-LIBS1 binding in these patients as the antibody recognizes a cryptic epitope that is exposed by the conformational changes that follow ligand binding be it fibrinogen, von Willebrand factor or small peptides. The reduced binding of anti-LIBS1 in uremic platelets therefore represents a true reduction in the active form of the fibrinogen receptor.

During dialysis, there was no alteration in the GPIIb-IIIa receptor density (CD41) or the functional state of the receptor (LIBS1) in the systemic circulation. Similarly, there was no increase in alpha granule protein expression (GMP140, CD62). This may not be consonant with the occurrence of platelet release reaction during dialysis, as evidenced by increased beta thromboglobulin in plasma [4]. This apparent discrepancy may be related to the fact that platelets with increased GMP140 surface expression are rapidly cleared by sequestration in the microvasculature. This may occur via adhesion molecules (selectins) either directly with the endothelium or by mediating platelet-leukocyte microaggregates [15]. Alternatively, platelet consumption may be occurring in the extracorporeal circuit with resultant increase in beta thromboglobulin [4], but the fragments of these consumed platelets are not detectable by FACS analysis. Quantitatively, the magnitude of platelet clearance is not reflected in total platelet counts in the systemic circulation which are minimally affected during the procedure. Platelet counts are an insensitive method to measure platelet consumption, particularly as it pertains to the formation of platelet-leukocyte coaggregates considering the large discrepancy in the circulating numbers of the two cell populations.

Several investigators have reported the detection of platelet-leukocyte co-aggregates in the circulation under normal and pathophysiologic states [5–10]. Whether this phenomenon is a true *in vivo* state or the result of *in vitro* alterations remains to be determined. However, the dependence of the formation of these co-aggregates on divalent cations and specific epitopes of the GMP140 molecule, and the reproducible changes observed with cardiopulmonary bypass [10] and across dialyzers in the present study, strongly indicate that it is a real *in vivo* phenomenon. Further, the induction of this phenomenon *in vitro* with the platelet specific agonist ADP in physiologic concentrations favors the consideration of this phenomenon as a true biologic event. Taken together, these observations are consistent with a true cell-to-cell aggregation. The theoretical possibility that part of the signal may be due to passive adhesion of either soluble CD41 or CD41 shed from platelets to leukocytes cannot be unequivocally excluded based on the methods used in the present study.

The mechanisms underlying the aggregation are unclear, but several possibilities can be advanced including roles for platelet GMP140, fibrinogen receptor, or other adhesion molecules. GMP140 is an integral membrane glycoprotein constitutively expressed in alpha granules of platelets [13]. *In vitro* studies have indicated that GMP140 on activated platelets mediates the binding of platelets to neutrophils and monocytes but not to lymphocytes [16–18]. Platelet-leukocyte aggregation mediated by GMP140 would be dependent on prior platelet degranulation required to release GMP140 which remains associated with the plasma membrane. The counterreceptor on leukocytes would be CD15 [19].

Others have advanced evidence supporting a role for GPIIb-IIIa in mediating platelet-leukocyte co-aggregation [20]. MAC1 expression on monocytes and neutrophils is up-regulated during hemodialysis [21, 22] and this integrin binds fibrinogen [23]. Fibrinogen ligand bridging between fibrinogen binding integrins on platelets (GPIIb-IIIa) and granulocytes (MAC1) [18] may therefore play a role in dialysis induced platelet-leukocyte aggregation. Further studies are necessary to evaluate this question. It is clear, however, that multiple mechanisms of co-aggregation may co-exist and the significance of each may depend on the activational state of the platelet and the leukocyte. GPIIb-IIIa is

expressed early in platelet activation and is a reversible phenomenon whereas GMP140 expression follows platelet degranulation. Initial coaggregation may thus be GPIIb-IIIa dependent and as the platelets degranulate they release GMP140 which further contributes to coaggregation.

Whatever the underlying mechanism for this phenomenon, our data show that uremic subjects have reduced basal platelet-leukocyte coaggregation that may be related to a defect in platelet adhesion molecules or the fibrinogen receptor if such interactions are mediated by the latter. There is increasing evidence for quantitative and qualitative defects in the fibrinogen receptor in uremia which may explain the reduced basal co-aggregation in ESRD subjects. In favor of such a role is the parallelism between the known reduced platelet LIBS1 response to ADP in uremia and the diminished anti-CD41 binding to leukocytes in response to ADP in uremic subjects found in the present study.

We have observed an increased formation of platelet-neutrophil and platelet-monocyte coaggregation (as reflected in surface expression of CD41 on these leukocytes) after passage through the dialyzer, but no increase in platelet-leukocyte interaction. The latter observation is not unexpected as platelet-leukocyte interaction is usually very minimal [5, 10, 14]. In general, the signal for platelet-monocyte co-aggregates has exceeded that for platelet-neutrophil co-aggregates. This is consistent with *in vitro* data showing a competitive advantage of monocytes over neutrophils for binding to activated platelets [5].

The mechanism underlying increased platelet-neutrophil and platelet-monocyte co-aggregates during passage through the dialyzer may be due to primary activation of platelets by shear stress, contact activation, or agonist activation. ADP-activation of platelet *in vitro* leads to a dose dependent increase in the formation of platelet-neutrophil and platelet-monocyte co-aggregates, whereas the addition of complement factors C3a and C5a which are increased during dialysis has no effect on this phenomenon. The reproduction of the phenomenon by ADP addition *in vitro* should not be construed to imply that ADP *per se* is responsible for the occurrence of the phenomenon of membrane receptor transfer occurring within the dialyzer. We used ADP as a specific primary platelet agonist to suggest that the phenomenon observed in dialysis is related to a primary platelet activation rather than secondary to complement activation. Whether primary leukocyte activation may contribute to the formation of these co-aggregates is not entirely ruled out at present.

The design of the present study was to choose synthetic membranes associated with minimal or no leukopenia to avoid the problem of leukocyte-subpopulation analysis. Had we chosen to study membranes with significant leukopenia, the phenomenon may have been obscured by the large pulmonary sequestration of co-aggregates. We did, however, evaluate a modified cellulosic membrane (Hemophan) with an attenuated leukopenic response compared to unmodified cellulose, and found the induction of platelet-leukocyte aggregates by this cellulosic membrane to be similar to the three synthetic membranes.

While there is a step up across the dialyzer in the formation of platelet-leukocyte co-aggregates, the level of these co-aggregates in the systemic circulation is not altered during dialysis. Several explanations may be advanced for this observation. The platelet-leukocyte co-aggregates may be sequestered in the microvasculature, the co-aggregation may be reversible and hence may dissociate in the systemic circulation, or that the percentage of leukocytes with platelet co-aggregates as a proportion of the systemic leukocyte pool is small and the signal is thereby diluted and undetected by the method employed.

The significance of co-aggregate formation resides in the mechanisms of cell cross-talk. Platelets, for example, supply free cholesterol to monocytes and the latter may become involved in foam cell generation in atherosclerotic plaques [5]. There is also an elaborate degree of cross-talk between neutrophils and platelets [6, 8]. In general, under resting conditions a reciprocal inhibitory effect predominates. When these cells are activated, reciprocal activation occurs thereby magnifying the response [6]. Neutrophils utilize platelet derived arachidonate to increase leukotriene and other eicosanoid synthesis, and neutrophil derived cathepsin G may induce further platelet aggregation and secretion [8]. Further, platelets and neutrophils can cooperate to increase the formation of platelet activating factor (PAF) which acts on both cell types and offers the potential for amplification of activation [8]. One serious clinical situation where platelet-neutrophil interactions have been implicated is in the pathophysiology of septic shock and multiple organ system failure [7, 8, 24]. It is intriguing to speculate, but remains to be determined, whether the formation of platelet-leukocyte co-aggregates during dialysis has any real pathophysiologic consequence in patients with end-stage renal disease.

To date, co-investigation of the interplay between the different parameters of biocompatibility has centered on plasmatic-cellular interactions (such as complement and neutrophils) [25–27]. The evaluation of platelet-leukocyte aggregates combined with characterization of surface expression of activated GPIIb-IIIa on platelets might be a useful concept for studying biocompatibility of dialyzer membranes. A study of platelet-leukocyte interactions would offer the novel aspect of cellular-cellular interactions as a possible parameter of hemocompatibility.

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References

1. Remuzzi G, Pusineri F: The uraemic platelet (Chapt 8), in *Hemostasis and the Kidney,* edited by Remuzzi G, Rossi EC, London, Butterworths, 1968, pp. 107–118
2. Hakim RM, Schafer AI: Hemodialysis-associated platelet activation and thrombocytopenia. *Am J Med* 78:575–580, 1985
3. Sultan Y, London GM, Goldfarb B, Toulon P, Marchais SJ: Activation of platelets, coagulation and fibrinolysis in patients on long term hemodialysis: Influence of cuprophan and polyacrilonitrile membrane. *Nephrol Dial Transplant* 5:362–368, 1990
4. Gawaz MP, Ward RA: Effects of hemodialysis on platelet derived thrombospondin. *Kidney Int* 40:257–265, 1991
5. Rinder HM, Bonan JL, Rinder CS, Ault KA, Smith BR: Activated and unactivated platelet adhesion to monocytes and neutrophils. *Blood* 78:1760–1769, 1991
6. Bazzoni G, Dejana E, Del Maschio A: Platelet neutrophil interactions. Possible relevance in the pathogenesis of thrombosis and inflammation. *Haematologica* 76:491–499, 1991

7. HEFFNER JE, SAHN SA, REPINE JE: The role of platelets in the adult respiratory distress syndrome: Culprits or bystanders. *Am Rev Resp Dis* 135:482–492, 1987
8. FAINT RW: Platelet-neutrophil interactions: Their significance. *Blood Rev* 6:83–91, 1992
9. MAEDA T, NASH GB, CHRISTOPHER B, PECSVARADY Z, DORMANDY JA: Platelet-induced granulocyte aggregation *in vitro*. *Blood Coag Fibrinolysis* 2:699–703, 1991
10. RINDER CS, BONAN JL, RINDER HM, MATHEW J, HINES R, SMITH BR: Cardiopulmonary bypass induces leukocyte-platelet adhesion. *Blood* 79:1201–1205, 1992
11. WARE JA, HEISTAD DD: Platelet endothelium interactions. *N Engl J Med* 328:628–635, 1993
12. GINSBERG MH, FRELINGER AL, LAM SCT, FORSYTH J, MCMILLAN R, PLOW EF, SHATTIL SJ: Analysis of platelet aggregation disorders based on flow cytometric analysis of membrane glycoprotein IIb-IIIa with conformation specific monoclonal antibodies. *Blood* 76:2017–2023, 1990
13. STENBERG PE, MCEVER RP, SHUMAN MA, JACQUES YV, BAINTON DF: A platelet alpha granule membrane protein (GMP140) is expressed on the plasma membrane after activation. *J Cell Biol* 101:880–885, 1985
14. RINDER HM, BONAN JL, RINDER CS, AULT KKA, SMITH BR: Dynamics of leukocyte platelet adhesion in whole blood. *Blood* 78:1730–1737, 1991
15. GINSBERG MH, LOFTUS JC, PLOW EF: Cytoadhesins, integrins, and platelets. *Thromb Haemost* 59:1–6, 1988
16. HAMBURGER SA, MCEVER RP: GPM140 mediates adhesion of stimulated platelets to neutrophils. *Blood* 75:550–555, 1990
17. LARSEN E, CELI A, GILBERT GE, FURIE BC, ERBAN JK, BONFANTI R, WAGNER DD, FURIE B: PADGEM protein: A receptor that mediates the interaction of activated platelets and monocytes. *Cell* 59:305–311, 1989
18. GAWAZ MP, LOFTUS JC, BAJT ML, FROJMOVIV MM, PLOW EF, GINSBERG MH: Ligand bridging mediates integrin (platelet GPIIb-IIIa) dependent homotypic and heterotypic cell-cell interactions. *J Clin Invest* 88:1128–1134, 1991
19. LARSEN E, PALABRICA T, SAJER S, GILBERT GE, WAGNER DD, FURIE BC, FURIE B: PADGEM-dependent adhesion of platelets to monocytes and neutrophils is mediated by lineage specific carbohydrate, LNFIII (CD15). *Cell* 63:467–484, 1990
20. SPANGENBERG P, REDLICH H, BERGMANN I, LOSCHE W, GOTZRATH M, KEHREL B: The platelet glycoprotein IIb/IIIa complex is involved in the adhesion of activated platelets to leukocytes. *Thromb Haemost* 70:514–521, 1993
21. ARNAOUT MA, HAKIM RM, TODD RF, DANA N, COLTON HR: Increased expression of an adhesion promoting surface glycoprotein in the granulocytopenia of hemodialysis. *N Engl J Med* 312:457–462, 1985
22. HIMMELFARB J, ZAOUI P, HAKIM R: Modulation of granulocyte LAM 1 and MAC1 during dialysis a prospective, randomized controlled trial. *Kidney Int* 41:388–395, 1992
23. ALTIERI DC, BADER R, MANNUCCI PM, EDGINGTON TS: Oligospecificity of the cellular adhesion receptor MAC1 encompasses an inducible recognition specificity for fibrinogen. *J Cell Biol* 107:18931–18940, 1988
24. GAWAZ MP, FATEH-MOGADAM S, PILZ G, WERDAN K, GURLAND HJ: Increased platelet thrombospondin expression and GPIIb-IIIa activation in multiple system organ failure. *Ann Hematol* 66(Suppl I):A62, 1993
25. CRADDOCK PR, FEHR J, BRIGHAM KL, KRONENBERG RS, JACOB HS: Complement and leukocyte mediated pulmonary dysfunction in hemodialysis. *N Engl J Med* 296:769–774, 1977
26. CRADDOCK PR, HAMMERSMITH DE, WHITE JG, DALMASSO AP, JACOB HS: Complement (C5a) induced granulocyte aggregation *in vitro:* A possible mechanism of complement mediated leukostasis and leukopenia. *J Clin Invest* 60:260–264, 1977
27. HAKIM RM, FEARON DT, LAZARUS JM: Biocompatibility of dialysis membranes: Effects of chronic complement activation. *Kidney Int* 26:194–200, 1984